Guided bone regeneration (GBR) using membranes and calcium sulphate after apicectomy: a comparative histomorphometrical study

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Abstract

Yoshikawa G, Murashima Y, Wadachi R, Sawada N, Suda H. Guided bone regeneration (GBR) using membranes and calcium sulphate after apicectomy: a comparative histomorphometrical study. *International Endodontic Journal*, **35**, 255–263, 2002.

Aim The purpose of the present study was to evaluate the effects of resorbable and non-resorbable membranes, and calcium sulphate on bone regeneration in osseous defects in conjunction with apicectomy.

Methodology The mandibular third and fourth premolars of 12 beagle dogs were root treated, and apicectomies were performed. The osseous defects were divided randomly into five groups. In groups A, B and C the osseous defects were covered with e-PTFE membranes, PLGA membranes, and collagen membranes, respectively. In group D, defects were filled with calcium sulphate. Nothing was used in group E, which served as controls. The dogs were sacrificed 4, 8, and 16 weeks after the surgery. Undemineralized sections were obtained and evaluated histomorphometrically.

Results Newly formed cortical bone had closed the defect in the cortical plate in all groups at 16 weeks. The degree of concavity of the new cortical bone at 16 weeks in groups A and D was significantly less than in group B ($P < 0.01$). The percentage of regenerated bone in group A was significantly greater than in groups B ($P < 0.01$), C ($P < 0.05$) and E ($P < 0.05$). In group D, it was significantly greater than in groups B ($P < 0.01$) and E ($P < 0.05$).

Conclusions The data suggests that e-PTFE membrane is more effective compared to resorbable membranes and controls for bone regeneration after apicectomy, and that calcium sulphate could be substituted for e-PTFE membrane.

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Keywords: apicectomy, calcium sulphate, guided bone regeneration, membrane.

Received 6 February 2001; accepted 20 April 2001

Introduction

Osseous defects following apicectomy can be large and complicated in cases with substantial apical lesions, such as combined endodontic-periodontal lesions and through-and-through osseous defects. Andreasen & Rud (1972) divided modes of healing after periapical surgery into two types: (i) healing by periodontal tissue regeneration, and (ii) healing by fibrous scar tissue. The most desirable healing after apicectomy is regeneration by bone tissue without scar tissue formation, comparable to healing by periodontal tissue regeneration.

Nyman *et al.* (1982) first reported the application of guided tissue regeneration (GTR) to periodontal defects in humans. The biological principle of GTR is to exclude dentogingival epithelium and gingival connective tissue proliferation into the wound area adjacent to the root surfaces and, simultaneously, to create a space to give preference to periodontal ligament cells for coronal migration (Nyman 1991). Guided bone regeneration (GBR) is the application of the concept of GTR to osseous defects. Several studies have shown that GBR using expanded polytetraflouroethylene (e-PTFE) membrane was successfully applied to osseous defects in conjunction with apicectomy (Dahlin *et al.* 1990, Pecora *et al.*

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1995). However, non-resorbable membranes, such as e-PTFE membrane, must be removed some time later following the initial surgery, whilst follow-up surgery is not necessary for resorbable membranes. Several types of resorbable membranes, including the polylactic acid-co-glycolic acid (PLGA) membrane and collagen membrane, have been introduced and applied to GTR (Tanner *et al.* 1988, Minabe *et al.* 1989, Magnusson *et al.* 1990, Kon *et al.* 1991, Caffesse *et al.* 1994, Gottlow *et al.* 1994, Cortellini *et al.* 1996). However, it has not been determined whether the application of resorbable membranes is effective for GBR or not (Simion *et al.* 1996, Uchin 1996, Ito *et al.* 1998, Maguire *et al.* 1998, Zahedi *et al.* 1998, Bohning *et al.* 1999).

As an alternative, calcium sulphate has recently been applied to osseous defects during apicectomy as a substitute for membranes (Pecora *et al.* 1997b). Membranes are difficult to apply in cases with no cervical cortical bone, and in cases with a through-and-through osseous defect, whilst the application of calcium sulphate to GBR is easier.

Although many studies have reported on resorbable and non-resorbable membranes, and calcium sulphate in periodontal treatment (Radentz & Collings 1965, Tanner *et al.* 1988, Minabe *et al.* 1989, Magnusson *et al.* 1990, Kon *et al.* 1991, Caffesse *et al.* 1994, Gottlow *et al.* 1994, Cortellini *et al.* 1996, Andreana 1998, Kim *et al.* 1998a,b), only a few studies have been made on bone regeneration in endodontics (Dahlin *et al.* 1990, Kellert *et al.* 1994, Pecora *et al.* 1995, 1997b, Uchin 1996, Maguire *et al.* 1998).

The purpose of the present study was to evaluate histomorphometrically the effects of resorbable and non-resorbable membranes, and calcium sulphate on bone regeneration in osseous defects in conjunction with apicectomy.

Materials and methods

Twelve beagle dogs were used in this study with the approval of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. The animals were sedated with an intramuscular injection of ketamine hydrochloride (0.3 mg kg^{-1}, Sankyo Co. Ltd., Tokyo, Japan), and subsequently anaesthetized with sodium-pentobarbital (12.5 mg kg^{-1}, Tanabe Seiyaku Co. Ltd., Osaka, Japan) given intravenously and added when necessary to maintain deep anaesthesia. In addition, local anaesthesia was performed with an infiltrative injection of 2% lidocaine with 1 : 80 000 epinephrine (Astra Japan Ltd., Osaka, Japan) to control haemorrhage.

Following the general and local anaesthesia, pulpectomies were performed on the mandibular third and fourth premolars on both sides. The root canals were instrumented with K-files (sizes 10–50) and Gates Glidden burs (sizes 2–4), and alternately irrigated with 6% sodium hypochlorite and 3% hydrogen peroxide. After the canals were dried with paper points, they were obturated with gutta-percha and root canal sealer by the lateral condensation method. The access cavities were sealed with glass-ionomer cement.

After the root canal treatments, full mucoperiosteal triangular flaps on both sides were reflected from the distal region of the first molar (vertical releasing incision) to the mesial region of the second premolar (intrasulcular incision). Cortical bone was removed using a trephine bur (4.8 mm in diameter) under water cooling, and the roots of the third and fourth premolars were resected with a fissure bur, also under water cooling. Subsequently, retrograde cavities were made using an ultrasonic tip (Osada Electric Co. Ltd., Tokyo, Japan). Following haemostasis of the osseous defects with 0.1% epinephrine pellets and drying the retrograde cavities with air, Super-EBA® (Harry J. Bosworth, Skokie, IL, USA) was placed as a retrofill. The osseous defects were randomly divided into five groups. In groups A, B, and C the osseous defects were covered with e-PTFE membranes (W. L. Gore & Associates, Inc., Flagstaff, AZ, USA), PLGA membranes (GC Corp., Tokyo, Japan), and collagen membranes (Koken Co. Ltd., Tokyo, Japan), respectively. In group D the defects were filled with medical grade calcium sulphate (Class Implant, Rome, Italy). In group E, they received no further treatment and served as controls. All mucoperiosteal flaps were repositioned and sutured.

The dogs were divided into three groups of four animals each, and they were allowed healing periods of 4, 8, and 16 weeks after surgery, respectively. During those periods, bone labelling with fluorescent dyes was completed as follows: tetracycline (20 mg kg^{-1}, Sigma, St. Louis, MO, USA) and calcein (8 mg kg^{-1}, Fluka, Buchs, Switzerland) were injected subcutaneously at 15 days and 2 days before sacrifice, respectively. The animals were sacrificed by an overdose of sodium-pentobarbital. Mandibular blocks including the third and fourth premolars were removed, sectioned into small segments containing a single root each, and immediately fixed with 8% formalin and 2.5% glutarardehyde solution for 1 week at 4°C. After fixation, each segment was dehydrated in a series of graded ethanol concentrations and embedded in polyester resin (Nisshin EM, Tokyo, Japan). Undemineralized semiserial sections in the buccolingual direction were obtained from the central portion of the root using

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Table 1 Morphometrical parameters

	Definitions	Unit
Measured parameters		
Bone volume (BV)	Total bone area within the measuring area	mm^2
Tissue volume (TV)	Measuring area	mm^2
Concavity of new cortical bone	Distance from the reference line to the most concave point of new cortical bone	mm
Calculated parameter		
BV/TV	Bone volume/tissue volume ×100	%

a diamond saw (Exact-Apparatebau, Hamburg, Germany) and ground to approximately 50 µm in thickness. The specimens were stained with toluidine blue stain or Villanueva's bone stain. Each section was evaluated both histologically and morphometrically under a light microscope and a fluorescence microscope.

Histological evaluation

Tissue sections were evaluated histologically with respect to: (i) presence of the membrane or calcium sulphate at each period; (ii) tissue reaction to the materials used; (iii) inflammation in the osseous defect area; (iv) bone regeneration into the osseous defect; (v) deposition of cementum-like structure, root resorption, and ankylosis on the resected root surface.

Morphometrical evaluation

An image analysing system (KS400®, Carl Zeiss Co. Ltd., Göttingen, Germany) was used to measure the morphometric parameters on the image, which was captured by computer software (Adobe Photoshop®, Adobe Systems Inc., San Jose, CA, USA). The images were numbered randomly to blind the experimental groups, and another examiner performed all the morphometrical evaluations to avoid measurement error.

The measured and calculated parameters are shown in Table 1. Four reference points (a, b, c, d in Fig. 1), which were represented by the outer and inner edges of the original cortical bone, clearly created the measuring area representing tissue volume (TV). Bone volume (BV) was defined as the total bone areas within the measuring area. BV/TV was calculated from the percentage of bone volume in the measuring area. The reference line (a–b) was drawn between the outer-top and outer-bottom edges of the original cortical bone (Fig. 1). Concavity of new cortical bone at 16 weeks was obtained by measuring the longest distance from the reference line to the most concave point of new cortical bone (Fig. 1). For

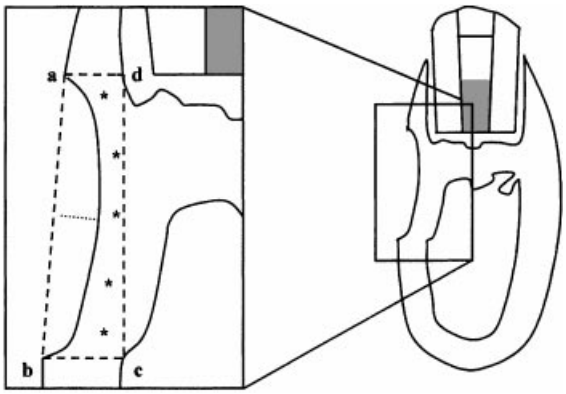

Figure 1 Diagram of morphometrical measurements. Trapeziform measuring area is surrounded by the broken lines. Bone volume is the area marked by asterisks. Dotted line, concavity of new cortical bone; a, b, c, d, outer and inner edges of original cortical bone; a–b, reference line.

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statistical analysis, the one-way ANOVA, and two-way ANOVA with Fisher's posthoc test were performed.

Results

Histological findings

At 4 weeks, osseous defects in all groups were filled with granulation tissue and newly formed woven bone that was labelled diffusely by tetracycline and calcein (Fig. 2). Calcium sulphate was not recognized at 4 weeks. Collapsing of the membranes into the osseous defects was not observed. In addition, no sign of tissue reaction to the membrane was observed in any membrane group. However, the internal portion of PLGA membrane was degraded, and cell infiltration linked to the resorption of the collagen membrane was observed (Fig. 3). No signs of root resorption or cementum-like structure on the resected root surface were found.

At 8 weeks in all groups, the new cancellous bone that had formed in the osseous defects was mature. In some

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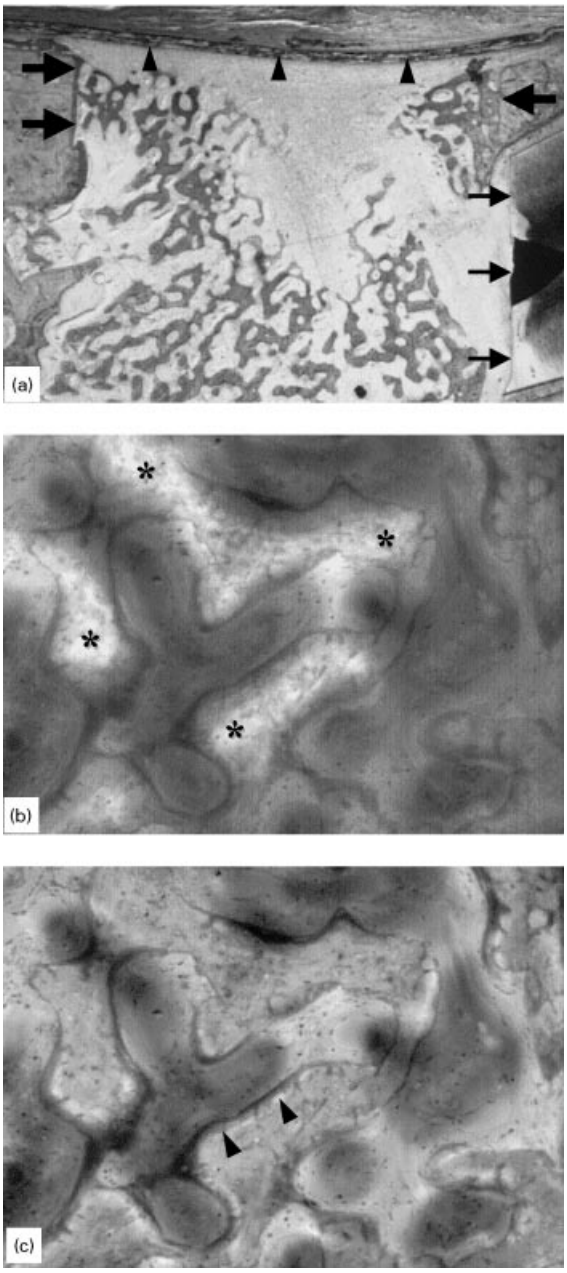

Figure 2 Representative sections of group A at 4 weeks after the surgery. (a) The osseous defect was being filled with newly formed woven bone. Large arrows, resected surfaces of original cortical bone; Small arrows, resected root surface; Arrowheads, e-PTFE membrane. (×2.5, Toluidine blue stain) (b, c) High magnifications of newly formed woven bone under a fluorescence microscope. Woven bone was labelled by tetracycline and calcein. Asterisks in (b), labelling lines by tetracycline; arrowheads in (c), labelling lines by calcein. (×10)

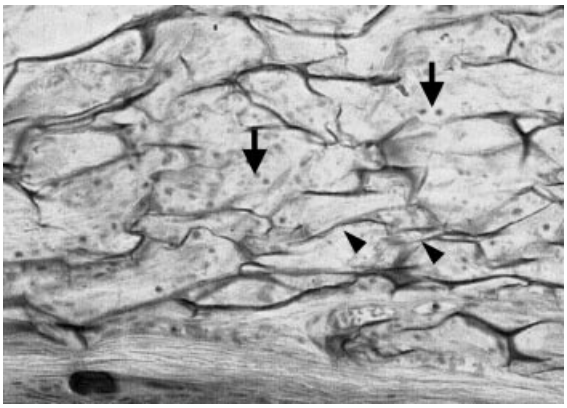

Figure 3 Representative section of group C at 4 weeks after the surgery. Arrowheads, sparse residues of collagen membrane; arrows, cell infiltration into the collagen membrane (×20, Toluidine blue stain).

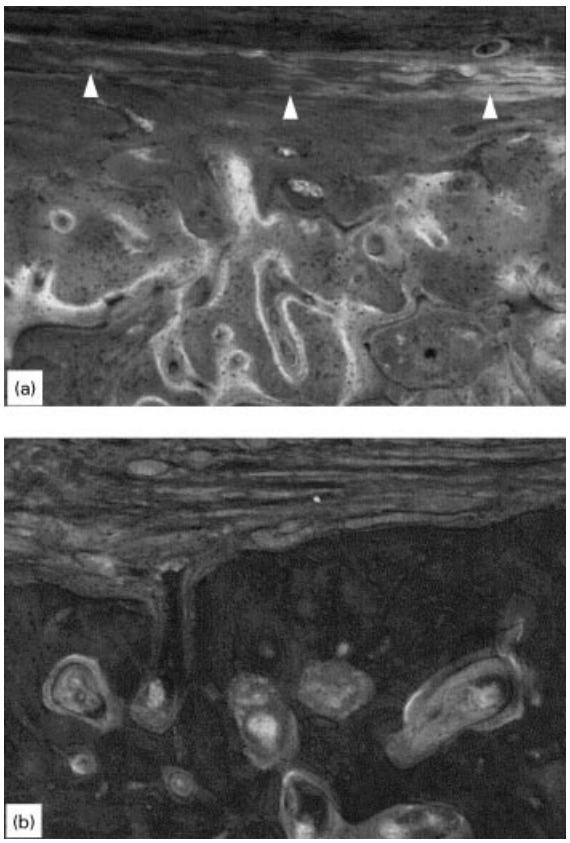

Figure 4 Newly formed cortical bone under a fluorescence microscope. (a) Eight weeks specimen of group A. Arrowheads, e-PTFE membrane. (×5) (b) Sixteen weeks specimen of group D. Calcium sulphate had been resorbed. (×5)

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Table 2 Concavity of new cortical bone at 16 weeks (mm)

Group	A	B	C	D	E
	0.50 ± 0.25 (5)	1.30 ± 0.58 (6)	0.85 ± 0.39 (5)	0.51 ± 0.12 (4)	0.74 ± 0.30 (3)

Mean value ± standard deviation (number).

specimens, the defects were closed by regenerated cortical bone that was clearly labelled by tetracycline and calcein (Fig. 4a). A meagre amount of bone was formed and a small number of inflammatory cells infiltrated adjacent to the root-end filling. Cementum-like structure was deposited on the resected root surface in some specimens except on the filling material. The PLGA membrane was more degraded than in the 4-week group. Sparse residues of the collagen membrane were observed.

At 16 weeks, newly formed cortical bone had closed the defect in the cortical plate in all groups (Fig. 5). Fluorescent lines, which labelled new cortical bone, were reduced compared with those at 8 weeks (Fig. 4b). Deposition of the cementum-like structure on the resected root surface was observed in almost all samples, and in some cases, fibre bundles were inserted into the newly formed cementum-like structure and bone. However, no such deposition was observed on the root-end filling.

Calcium sulphate had disappeared completely at 16 weeks. However, remnants of the collagen membrane were scattered. Traces of PLGA membrane were still visible, and collapsing into the bone defect was observed. The regenerated bone was observed in contact with the e-PTFE membrane and remnants of the collagen membrane. However, fibrous tissue between the PLGA membrane and the new cortical bone was thicker compared with the e-PTFE membrane and collagen membrane (Fig. 6).

Although root resorption on the resected surface was observed in some samples of all groups at 8 and 16 weeks, the cementum-like structure was formed in root resorption lacunae in almost all samples. There was no evidence of ankylosis on the resected surfaces during any of the periods in this experiment.

Morphological analysis

The results of concavity of the new cortical bone and BV/TV are shown in Tables 2 and 3, respectively. The degree of concavity of the new cortical bone was slight in groups A and D, but marked in group B (Fig. 5). There were significant differences between groups A and B ($P < 0.01$), and between groups B and D ($P < 0.01$) using the one-way ANOVA test (Table 2). BV/TV in group A was significantly higher than in groups B ($P < 0.01$), C ($P < 0.05$) and E ($P < 0.05$) when using the two-way ANOVA test. Also, BV/TV in group D was significantly higher than in groups B ($P < 0.01$) and E ($P < 0.05$) using the two-way ANOVA test (Table 3).

Table 3 BV/TV in each group at three observation periods (%)

Group	4 weeks	8 weeks	16 weeks
A	23.1 ± 6.61 (6)	36.5 ± 20.2 (5)	54.8 ± 16.9 (6)
B	17.6 ± 16.3 (7)	19.2 ± 7.30 (7)	21.2 ± 11.9 (8)
C	24.8 ± 14.9 (6)	21.7 ± 9.91 (7)	34.0 ± 15.6 (8)
D	17.4 ± 8.90 (3)	40.0 ± 15.5 (3)	48.9 ± 15.7 (4)
E	24.8 ± 10.3 (3)	12.8 ± 8.74 (4)	37.4 ± 11.9 (4)

Mean value ± standard deviation (number).

Discussion

The process and modes of healing in the osseous defect were similar histologically in all groups at each period. The histological findings using a light microscope and a fluorescence microscope showed that bone regeneration occurred diffusely about 2 weeks after the surgery. At 8 weeks, the volume of new bone labelled by fluorescence dyes in the osseous defect was greater than that at 16 weeks. Namely, the activity of bone regeneration at 8 weeks was higher than that at 16 weeks. The osseous defects were closed by newly formed cortical bone in all specimens at 16 weeks, and it appeared that bone remodelling activity in the osseous defect at 16 weeks was close to normal.

Many studies have reported that e-PTFE membrane is an excellent material for GTR in periodontal treatment (Kon *et al.* 1991, Caffesse *et al.* 1994, Cortellini *et al.* 1996). Also, several studies have shown that GBR using e-PTFE membrane is an effective technique because of its high ability to create a secluded space (Dahlin *et al.* 1989, 1990, Nyman 1991, Pecora *et al.* 1995, Simion *et al.* 1996, Ito *et al.* 1998). Dahlin *et al.* (1990) was the first to report in endodontics that bone regeneration using e-PTFE membrane was predictably achieved in osseous defects. Pecora *et al.* (1995) reported in a clinical study that GTR principle using e-PTFE membrane could be applied effectively to the healing of large periapical lesions. Simion *et al.* (1996) and Ito *et al.* (1998) compared the bone regeneration histologically when resorbable

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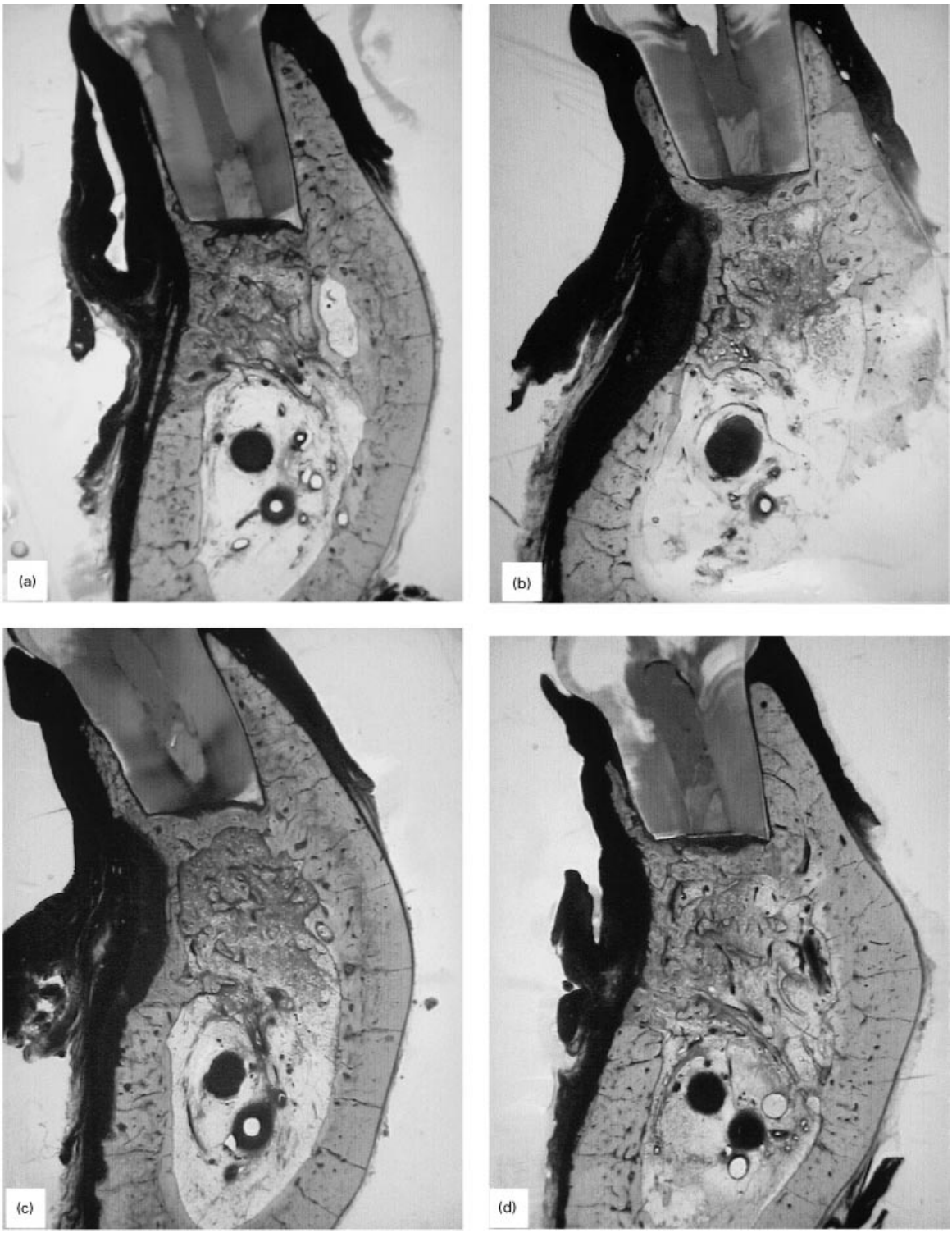

Figure 5 Representative sections at 16 weeks. Group A, e-PTFE membrane; group B, PLGA membrane; group C, collagen membrane; group D, calcium sulphate; group E, controls (×1, Villanueva's bone stain).

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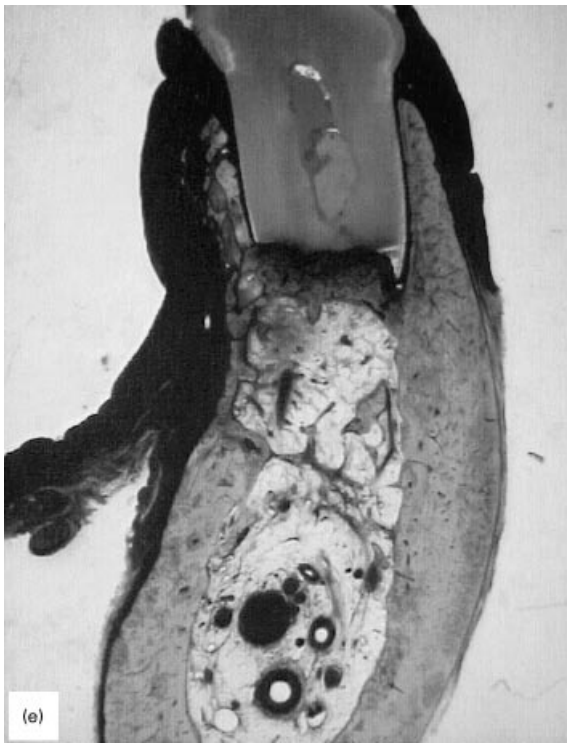

Figure 5 *continued.*

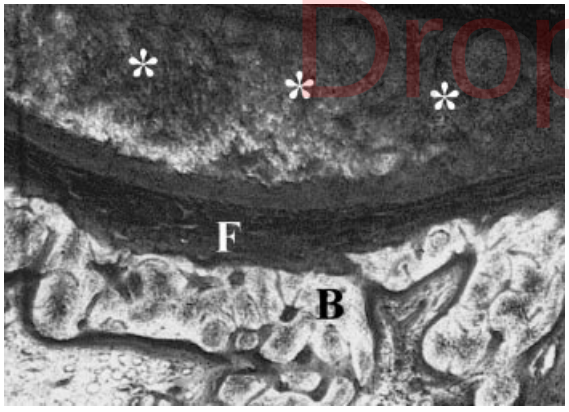

Figure 6 Representative section in group B at 16 weeks after the surgery. Asterisks, the trace of PLGA membrane; F, fibrous tissue; B, newly formed cortical bone (×5, Villanueva's bone stain).

and non-resorbable membranes were applied to osseous defects. They concluded that e-PTFE membrane was most effective for GBR. In the present study, the percentage of bone volume (BV/TV) using e-PTFE membrane was significantly higher, and the concavity of new cortical bone using e-PTFE membrane was smaller than that in controls. As such, the application of e-PTFE membrane to the osseous defect in conjunction with apicectomy was an effective technique to achieve bone regeneration.

In periodontology, Caffesse *et al.* (1994) and Cortellini *et al.* (1996) reported that similar findings could be achieved in GTR procedures when PLGA membrane and e-PTFE membrane were applied. However, Simion *et al.* (1996) reported that PLGA membrane produced some bone regeneration when compared with controls, but to a lesser extent than e-PTFE membrane. In the present study, PLGA membrane was inferior to e-PTFE membrane and not superior to controls in bone regeneration. The fibrous tissue between the PLGA membrane and the new cortical bone was thick, so that the new cortical bone in the PLGA membrane group was remarkably concave compared with the other groups. Possibly, the PLGA membrane was less biocompatible than e-PTFE membrane, collagen membrane, and calcium sulphate.

Collagen is known to show different effects on tissue healing depending on its type, structure, condition of cross-linking and the type of chemical treatment it has undergone. Furthermore, in GTR, microfibrillar collagen membrane, purified from bovine corium collagen, did not effectively prevent apical migration of epithelium in humans (Tanner *et al.* 1988). However, Zahedi *et al.* (1998) reported that diphenylphosphorylazide (DPPA), crosslinked collagen membrane, had physico-chemical characteristics compatible with the requirements for GBR. The collagen membrane used in this study was made from atelocollagen that was type I collagen, purified from bovine dermis and solubilized with pepsin. Minabe *et al.* (1989) reported that the use of atelocollagen membrane in periodontal wounds suppressed the epithelial down-growth along the root surfaces, which rapidly reduced postoperative inflammatory reaction and foreign body giant cell reaction compared with controls. In the present study, however, atelocollagen membrane was not effective for bone regeneration after apicectomy compared with controls.

The resorbable membranes used in this study were not effective for bone regeneration compared with e-PTFE membrane, and not different from controls. Another resorbable membrane, polylactic acid (PLA) membrane, has been applied in GTR (Magnusson *et al.* 1990, Gottlow *et al.* 1994) and GBR (Uchin 1996, Maguire *et al.* 1998, Ito *et al.* 1998, Bohning *et al.* 1999). Uchin (1996) indicated that the GBR procedure using PLA membrane was an effective technique as an adjunct to endodontic surgery. However, Bohning *et al.* (1999) found no statistically significant difference in bone regeneration in rat calvaria, whether the GBR using PLA membrane was

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introduced or not. Maguire *et al.* (1998) reported that the use of PLA membrane did not show a significant positive effect on periradicular osseous healing in cats. Explanations for these findings include: (i) the resorption period of the resorbable membranes might be too short for bone regeneration; (ii) the resorbable membranes might not have sufficient stiffness to prevent them from collapsing into the osseous defect; and (iii) resorption of membrane might adversely affect bone regeneration.

Calcium sulphate has been applied to osseous defects (Peltier 1961, Calhoun *et al.* 1965, Pecora *et al.* 1997a,b). This material is inexpensive, easy to apply, biocompatible, and completely resorbable. Membranes should be trimmed to cover at least 2–3 mm beyond the margins of the osseous defect and be totally submerged under the repositioned flap to minimize postoperative infection risk (Pecora *et al.* 1997b). This makes application of the membranes complicated. However the application of calcium sulphate is not difficult, because it is simply mixed and plugged into the osseous defect. Studies reported that the resorption of calcium sulphate and subsequent regeneration of bone occurred rapidly over a period of weeks or months (Peltier 1961, Pecora *et al.* 1997a, Kim *et al.* 1998a,b). Yamazaki *et al.* (1988) suggested that the resorption period of calcium sulphate might be related to its density. In the present study, calcium sulphate was resorbed at 4 weeks. This was similar to the previous report (Pecora *et al.* 1997a). Bone regeneration using calcium sulphate was similar to that using e-PTFE membrane and superior to controls in the present study. Although many studies reported that the application of calcium sulphate achieved good bone regeneration, its true mechanism is unclear (Peltier 1961, Calhoun *et al.* 1965, Pecora *et al.* 1997a). Yamazaki *et al.* (1988) reported that calcium sulphate did not induce bone formation in the femoral muscle pouch in mice at 6 weeks after implantation of calcium sulphate. Possibly, the calcium sulphate may not be osteoinductive but osteoconductive.

In the present study, controls were inferior to e-PTFE membrane and calcium sulphate with respect to the bone volume. Without bone regeneration after apicectomy, the prognosis of the tooth might be compromised because of insufficient resistance against occlusal forces and possible periodontitis in the long term. Also, if the contours of the alveolar bone are concave when the teeth are extracted, prosthodontic procedures or treatment with dental implants might be more difficult to perform. Therefore, it is important to regenerate bone tissue into the osseous defect in conjunction with apicectomy to accomplish predictable treatments.

Conclusions

The findings of the present study showed that e-PTFE membrane was most effective for bone regeneration into osseous defects in conjunction with apicectomy; that the resorbable membranes used were not effective compared with controls; and, that calcium sulphate could be substituted for e-PTFE membrane. However, additional experiments are required to determine whether or not calcium sulphate is effective for complicated types of osseous defect, such as combined endodontic-periodontal lesions and through-and-through osseous defects.

Acknowledgements

This study was supported by 'Research for the Future' Program from The Japan Society for the Promotion of Science (JSPS-RFTF 96I00205).

We are deeply grateful to Professor Keiichi Ohya (Pharmacology, Department of Hard Tissue Engineering, Division of Bio-Matrix, Graduate School, Tokyo Medical and Dental University) for his helpful advice.

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